

A PHYTOCHEMICAL STUDY OF ALLIUM SATIVUM – W.S.R TO ALLICIN CONTENT

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ABSTRACT

Allium sativum has attracted the interest of many researchers due to its wide range of therapeutic effects with minimal adverse reactions. Its role in promoting the female reproductive health can be well understood from the fact Acharya Kashyap in his text has described a full chapter *Lashuna Kalpadhyaya* mentioning that the woman consuming *Lashuna* will not suffer from diseases of *kati*, *shroni* (*pelvis*), *gramyadharma janya rogas* (sexually transmitted diseases) and infertility. Its effects are mainly attributed to its chemical constituents like Allicin, Ajoene and certain other sulphur compounds etc. In the present study, bulbs of *Allium sativum* were dried and in controlled temperature and fine powder was made. It was filled in

capsules and clinical trial was done in the patients of Hypomenorrhoea. In context of this, a phytochemical study of dried powder of *Allium sativum* was done and various chemical constituents of garlic have been investigated to support its pharmaco-therapeutic actions as per clinical study. As Allicin is the main alkaloid present in garlic, special attention was given to it. As the concentration of Allicin varies according to methods of processing, its loss on drying was assessed by the phyto-chemical analysis.

KEYWORDS: *Lashuna* (*Allium sativum*), Garlic, Allicin, Thio-2-propene-1-sulfinic acid S-allyl ester, Alliin, Ajoene, phytochemical analysis, Yamuna Safed (G-1).

1. INTRODUCTION

Allium sativum i.e *Lashuna* is a bulbous herb which has been used in India as a food item, spice and medicine since centuries. Garlic is one of the earliest cultivated plants, which is well mentioned in the Bible and in the literature of Ancient Israel (The Talmud), Egypt (Codex Ebers) and India (Vedas and Purans, Charak Samhita). Chinese people strongly believe that Garlic prolongs longevity.^[1]

The role of garlic in prevention of cardiovascular diseases, dyslipidemia, cancer etc. is well known. Also its insecticidal, nematicidal, rodenticidal and antifungal activity has been acclaimed by many researchers. *Acharya Charaka* has asserted that each and every substance present on our earth is useful in combating illness when applied with proper planning for a specific purpose (Shastri Kashinath and Chaturvedi Gorakhnath, 1989).

CHEMISTRY

The main chemical constituent of intact garlic is the amino acid Alliin, an alkyl derivative of cysteine alkyl sulfoxide, which may varies from 0.2 to 2.0% fresh weight.^[2] On an average, garlic cloves contain ~0.8% Alliin. A pure dehydration process, with no loss of ingredients, would result in a 2–2.5 mg/g Alliin content in the powder. However, garlic powders contain only 1% Alliin at most, indicating that more than half of the Alliin is lost during dehydration. The efficacy of its chemical constituents chiefly depends on the processing involved in preparation of its various forms like powder, extract and oils etc. Crushing, chewing or cutting (or exposing dehydrated, pulverized garlic to water) of garlic cloves release the vacular enzyme Allinase that rapidly lyses the cytosolic cysteine sulfoxide^[3] to form sulfenic acid (R-SOH)^[4] which immediately condenses to form Allicin: the compound which produces the odor of freshly cut garlic. Garlic contains at least 100 sulfur-containing compounds basic to medicinal uses.^[5] Allicin represents 70-80% of the total thiosulfinates. Other thiosulfinates formed include allyl-ss(o)-methyl (6-16% of total), methyl-ss(o)- allyl (3-9% of total), trans-1-propenyl (0.2-0.4% of total), trans-1-propenyl-ss (o)-methyl+methyl-ss(o)- trans-1-propenyl (0.1-2.5% of total) and methyl-ss(o)methyl (2% of total) thiosulfinates.^[6]

The thiosulfinates released from crushed garlic are reactive molecules and undergo a number of transformations, depending on the temperature, pH and solvent conditions. Allyl-s-thiosulfinates (Allicin) are the least stable of the eight thiosulfinates.^[4] The principal

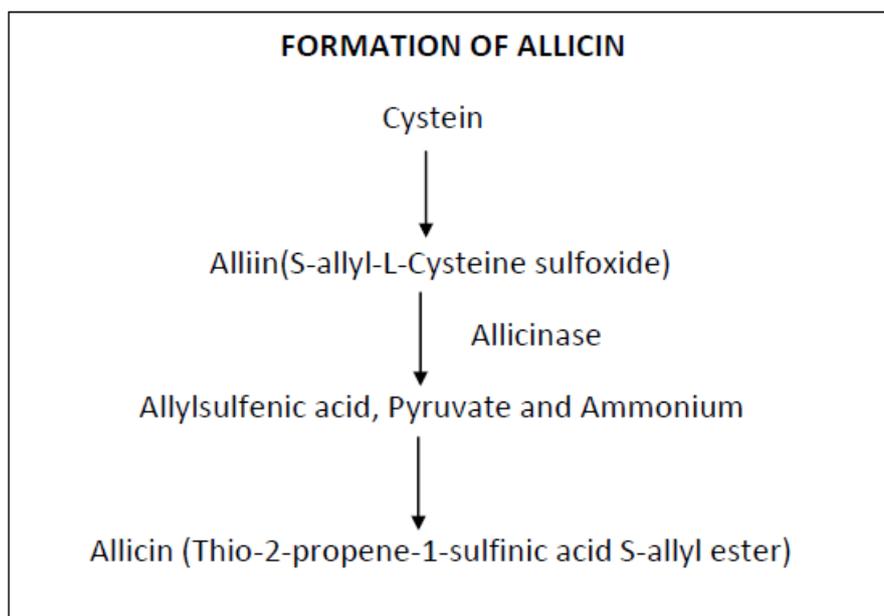
transformation products after incubation of thiosulfinate in water are diallyl trisulfide, diallyl disulfide and allyl methyl trisulfide.^[4]

ALLICIN

The health benefits related to garlic have been attributed to sulphur compounds particularly to thiosulfonates and among these substances, Allicin (diallylthiosulfinate) is the most abundant which is found in fresh garlic (approximately 0.4% by fresh mass).^[7]

Allicin is a product of enzymatic reaction when raw garlic is crushed or chopped. The enzyme, Allinase, stored in a separate compartment in garlic, combines with a compound called Alliin and produces Allicin. However, the preservation of Allicin is difficult as it is very unstable and reactive in nature. Once it is generated, it readily changes into other compounds such as allylsulfides, vinylidithins, and ajoenes.^[8]

Crushed raw garlic is high in Allicin, containing ~3.7 mg/g). Although Allicin is often emphasized in dehydrated powder, many preparations contained no Allicin, possibly reflecting its instability.



STANDARD PARAMETERS AS PER AYURVEDIC STANDARDS

As per Ayurvedic Pharmacopia of India Part-1, Volume -3, the following standards should be followed to ascertain the purity and efficacy of *Allium sativum*.

IDENTITY, PURITY AND STRENGTH

Foreign matter	Not more than 2 Per cent
Total ash	Not more than 4 Per cent
Acid-insoluble ash	Not more than 1 Per cent
Alcohol-soluble extracts	Not less than 2.5 Per cent
Water-soluble extracts	Not less than 60 Per cent
Volatile Oil	Not less than 0.1 Per cent

THIN LAYER CHROMATOGRAPHY (T.L.C.)

T.L.C. of the alcoholic extract on Silica gel 'G' plate using n-Butanol: Isopropanol Acetic acid: Water (3 : 1: 1 : 1) shows under UV (366 nm) two fluorescent zones at Rf. 0.58 and 0.72 (both light Blue). On exposure to Iodine vapour nine spots appear at Rf. 0.18, 0.26, 0.34, 0.38, 0.46, 0.58, 0.72, 0.77 and 0.93 (all Yellow). On spraying with Ninhydrin reagent and heating the plate for ten minutes at 110°C seven spots appear at Rf. 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all Pink). On spraying with Vanillin-Sulphuric acid reagent and heating the plate for ten minutes at 110°C seven spots appear at Rf. 0.26, 0.38, 0.46, 0.58, 0.67, 0.72 and 0.93 (all Grey).

CHEMICAL CONSTITUENTS

Volatile Oil contains Allyl Disulphide and Diallyl Disulphide. It also contains Allin, Allicin, Mucilage and Albumin.

PROPERTIES AND ACTION

RASA	:	Madhura, Katu
GUNA	:	Guru, Picchila, Sara, Snigdha, Tikshana
VIRYA	:	Ushna
VIPAKA	:	Katu
KARMA	:	Balya, Cakshushya, Deepana, Hridya, Kaphahara, Medhya, Raktadosha hara, Vata hara, Vrishya, Varnya, Pitta dushanakara, Bhagnasandhanakara, Rasayana, Jantughna, Kantha and Asthi Mansa Sandhanakara

2. MATERIALS AND METHODS

2.1 Materials

Fresh garlic bulbs were purchased for the purpose of this study from the local market near, Gopabandhu Ayurveda mahavidyalaya, Puri, Orisa, India. These were later identified as Yamuna Safed (G-1) variety.

2.2 Processing

The raw bulbs were manually peeled off after immersion in water at room temperature for 30 minutes. These garlic cloves hence obtained were weighed and put in stainless steel tray and dried in an oven. The temperature of the oven was set at a low at 40⁰C. The process was continued for a period of 72 hrs with 8 hours at 40⁰C and the rest at room temperature. After complete drying, the garlic cloves were ground and strained through a fine mesh sieve to obtain the fine powder. After processing, the garlic powder obtained was filled in capsules and stored in containers at room temperature.

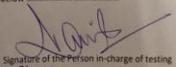
2.3 Determination of Allicin

Allicin concentration was determined in the prepared Garlic powder .Analysis was done at Satiat Research and Anatech Pvt. Ltd. (Approved by Food and Drug Administration, Haryana) GLP certified testing laboratory and Government Ayurvedic State Drug Testing Laboratory, Kurukshetra, Haryana, India.

3. OBSERVATION: The Findings were as following-

SR.NO	PROTOCOL	OBSERVATION
1.	Texture	Brown capsule containing Yellow powder
2.	Colour	Yellowish powder in side capsule
3.	Odour	Strong garlic odour
4.	Taste	Spicy
5.	pH	6.2
6.	Water soluble extract	70.06%
7.	Moisture content	11.18%
8.	Acid soluble ash	0.49%
9.	Total phenolic content	0.71
10.	Total ash	5.44%
11.	Physical Microscopy and Staining test	No viable organism, animal tissue or insect body part is detected in the sample

Allicin Content in prepared Garlic powder-

Allicin Content	3.37mg/gram																																																																																								
<div style="text-align: center;">  <p>GOVERNMENT AYURVEDIC STATE DRUG TESTING LABORATORY (In the Campus of Sh. Krishna Govt Ayurvedic college, Umari Road, Kurukshetra) E-MAIL:- sdtiharyana@gmail.com</p> </div> <p style="text-align: center;">Certificate of Analysis</p> <p>Drug License No. : S-ISM (HR) DTL Certificate No.: Allium Sativum/17042017/001</p> <p style="text-align: center;">FORM 13A [See rule 163 (5)]</p> <p>Certificates of tests or analysis by Government Analyst/ Person in-charge of testing under section 33H of the Drugs and Cosmetics Act, 1940</p> <ol style="list-style-type: none"> Names, from whom received : Dr. ANJANA TAK Ph.D Scholar, Gam puri, Utkal University, Orissa Serial No and date of memorandum no. : ---NA--- Name of sample : Allium Sativum (Lashuna) Dried Powder in Cap. Date of receipt : 17/04/2017 Names of ingredients purporting to have been used in the preparation of the sample..NA Condition of seals on the package...Intected Results of test or analysis <table border="1"> <thead> <tr> <th>Sr.no.</th> <th>Protocol</th> <th>Observation</th> <th>Specification / Remarks</th> </tr> </thead> <tbody> <tr> <td>1.</td> <td>Uniformity of Weight</td> <td>500 mg</td> <td>500 mg average</td> </tr> <tr> <td>2.</td> <td>Texture</td> <td>Brown capsule containing yellow powder</td> <td></td> </tr> <tr> <td>3.</td> <td>Colour</td> <td>Yellowish powder in side cap.</td> <td></td> </tr> <tr> <td>4.</td> <td>Odour</td> <td>Strong Garlic odour</td> <td></td> </tr> <tr> <td>5.</td> <td>Taste</td> <td>Spicy</td> <td></td> </tr> <tr> <td>6.</td> <td>pH</td> <td>6.2</td> <td></td> </tr> <tr> <td>7.</td> <td>Water Soluble Extract</td> <td>70.06 %</td> <td></td> </tr> <tr> <td>8.</td> <td>Moisture Content</td> <td>11.18%</td> <td></td> </tr> <tr> <td>9.</td> <td>Acid Insoluble Ash</td> <td>0.49 %</td> <td></td> </tr> <tr> <td>10.</td> <td>Total Phenolic Content</td> <td>0.71</td> <td></td> </tr> <tr> <td>11.</td> <td>Total Ash</td> <td>5.44 %</td> <td></td> </tr> <tr> <td>12.</td> <td>Physical Microscopy and Staining Test</td> <td>No. viable organism, Animal tissue Or insect body part is detected in the sample.</td> <td>Absent</td> </tr> </tbody> </table> <p>Results of test or analysis with protocols of test or analysis applied as per Ayurvedic, Siddha, Unani Pharmacopoeial or in house developed standards.</p> <p>In the opinion of the undersigned, the sample referred to above is of standard quality/is not of standard quality as defined in the Act or the rules made thereunder for the reasons given below</p> <p>   Analyst Analyst Date..... Date..... </p> <p style="text-align: right;">  Signature of the Person in-charge of testing In-Charge Govt. Ayurvedic State Drug Testing Laboratory Sh. Krishna Govt. Ayurvedic College & Hospital Kurukshetra, Haryana </p>	Sr.no.	Protocol	Observation	Specification / Remarks	1.	Uniformity of Weight	500 mg	500 mg average	2.	Texture	Brown capsule containing yellow powder		3.	Colour	Yellowish powder in side cap.		4.	Odour	Strong Garlic odour		5.	Taste	Spicy		6.	pH	6.2		7.	Water Soluble Extract	70.06 %		8.	Moisture Content	11.18%		9.	Acid Insoluble Ash	0.49 %		10.	Total Phenolic Content	0.71		11.	Total Ash	5.44 %		12.	Physical Microscopy and Staining Test	No. viable organism, Animal tissue Or insect body part is detected in the sample.	Absent	<div style="text-align: center;">  <p>Satiata Research & Anatech Pvt. Ltd. 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Total liability of our institute is limited to the invoiced amount. This report is not to be reproduced wholly or in part and cannot be used as evidence in the court of law and should not be used in any advertising media without our special permission in writing. Sample drawn and submitted by the party for Analysis unless otherwise stated. <p style="text-align: center;"> Plot No. 264, 1st & 2nd Floor, HSIIDC, Barwala - 134118, Panchkula, Haryana. Ph. 01733-256 570, E-mail: satiateresearch@gmail.com, info@sralabs.com, website: www.sralabs.com </p>	Name of Sample	: Allium Sativum (Lahsuna) Dried powder capsules	A.R. No.	: SA/FP-170417/052	Batch No.	: N.M.	Batch size	: N.M.	D/M	: N.M.	D/E	: N.M.	Original Manufacturer's	: N.M.	Sample submitted by	: Dr. Anjana Tak, Ph. D. Scholar, Gam Puri, Utkal University, Orissa	Mfg. Lic. No	: N.M.	Ref. No. (As in test req. Slip)	: N.M.	Sample Qty	: 20 Capsules	Ref. No. (As given by party)	: N.M.	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4. DISCUSSION

EFFECT OF HEAT AND DRYING ON ALLICIN CONTENT

The efficacy of garlic and garlic products is directly related to the Allicin content. Many studies have been conducted to study the Allicin content of garlic samples dried by hot air-drying and freeze-drying were compared with the Allicin content of fresh garlic. In a study conducted by C. Ratti et al, 2007^[9], it was noticed that the average Allicin content in fresh garlic was found to be 0.3% of the wet garlic approximately. Allicin values after 40 and 50°C hot air-drying were close to those for fresh garlic. Moderate air temperatures (40 and 50°C) allowed a better Allicin retention than a higher temperature (60°C). It is clear from this data that the Allicin content decreases with an increase of hot air-drying temperature.

It was further noticed that if in hot air-drying temperature is more than 60°C, Allinase may get partly inactive or denatured and the remaining capacity to produce Allicin is therefore reduced. Statistics confirmed that Allicin content of garlic slices was significantly affected ($p < 0.05$) by hot air-drying temperature.

Freeze-dried whole cloves at 20°C presented a higher Allicin content (about 14%) than fresh garlic. This may be due to the differences in the sample preparation for HPLC analysis between fresh and dried garlic samples. However, the fact that whole cloves freeze-dried at 20°C showed the highest Allicin content when compared to other treatments may be due not only to the low temperatures during freeze-drying but also due to the fact that no cutting of the sample was done. Therefore, no Allicin was formed and lost during sample preparation as in the case of garlic slices.

In the present study Garlic powder was prepared by cutting the peeled raw garlic cloves into small pieces and drying them in shade. This allowed Alliinase to transform all the Alliin present in the pieces to Allicin and other Allyl thiosulfinates.

As in this present study Allicin content was 3.37mg/gram about 0.33%, it seems to be less than the expected value. It might be because of the dehydration technique or the time gap between drug manufacturing and analysis.

PROBABLE REASON OF ALLICIN LOSS AFTER DRYING

Previous works on the loss of Pyruvic acid during air drying of garlic^[10] stated that partial inactivation of the enzyme Allinase and destruction of precursors could be the cause for loss of pungency in garlic. Both Allicin and Pyruvic acid are the result of the Alliin-Allinase reaction; therefore, the loss of Allicin could be explained in a similar way.

Other probable cause may be that if the products are exposed to high temperatures for long times, and the internal structure is significantly damaged during the process. Thus, part of the Allinase enzyme could be inactive, or the Allicin formed during the process can be decomposed in disulfides or vinylidithiins with a subsequent loss through evaporation.^[10]

5. CONCLUSION

Though due importance is given to Allicin and it is held responsible for significant biological effects on cardiovascular, immune and hepato-biliary system; but the findings in this study indicates that it is not necessary to retain Allicin for its favorable effects. So, it can be inferred that some other compounds (Sulphur, non sulphur compounds, Saponins, Protein fractions etc) might be actually responsible for its health benefits. More researches are needed from different perspectives regarding the biological activities and action mechanisms, along with the bioavailability analysis.

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